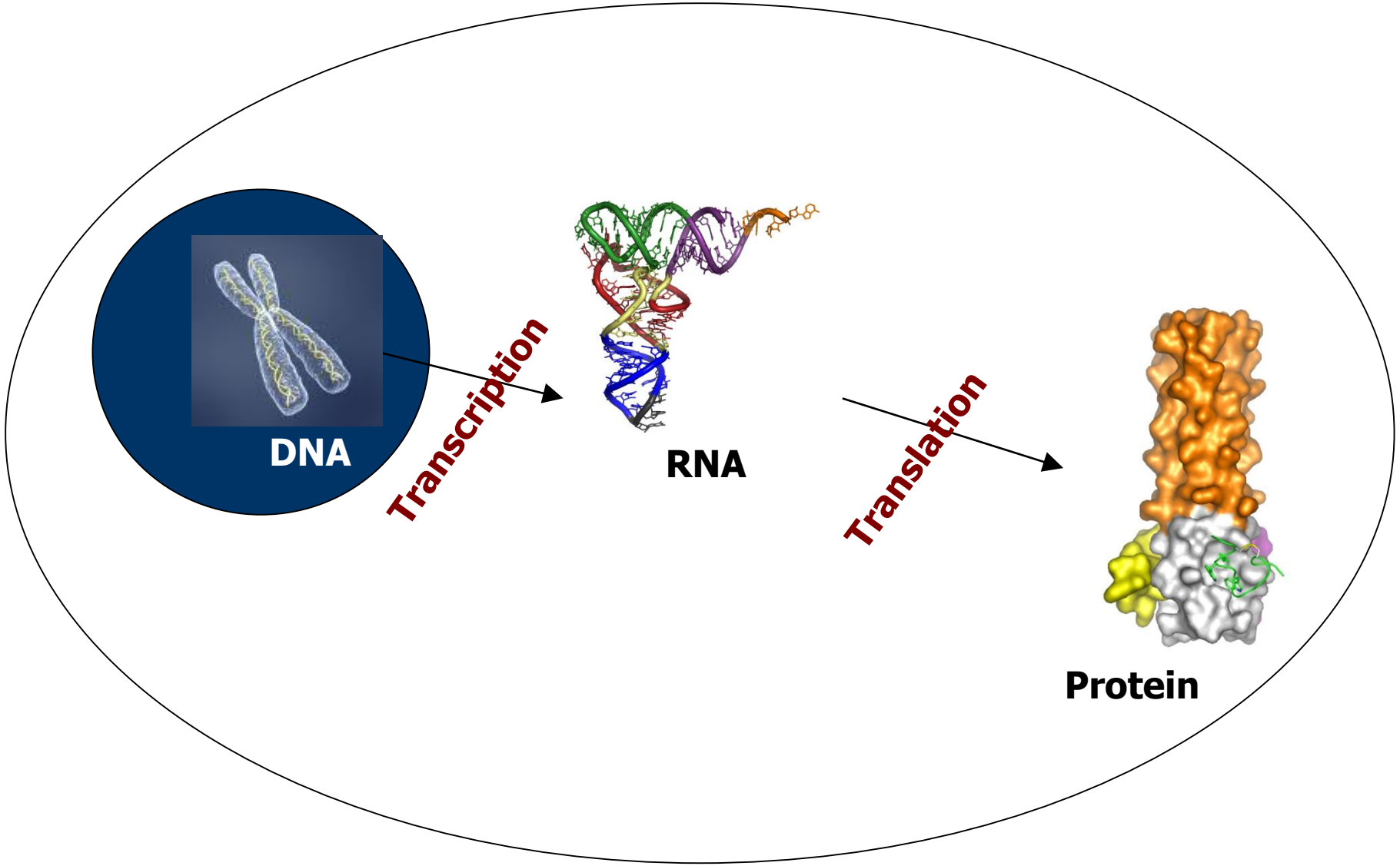
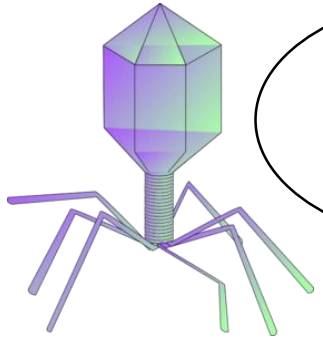
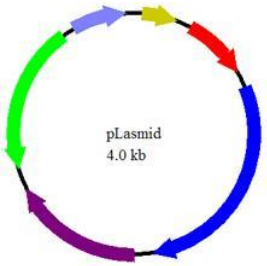
Transformation of GFP

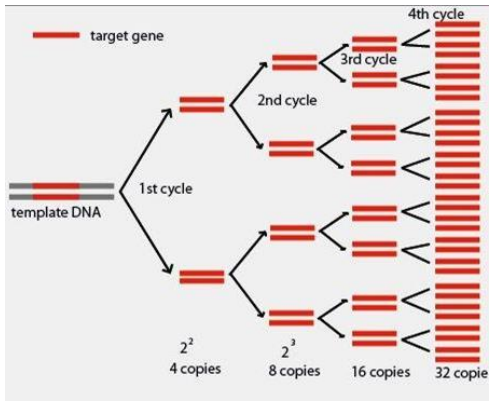
Protein Production- Reminder

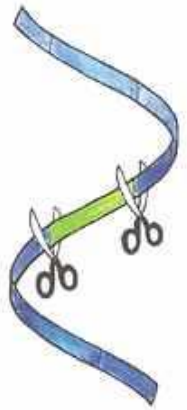


How is genetic engineering done?



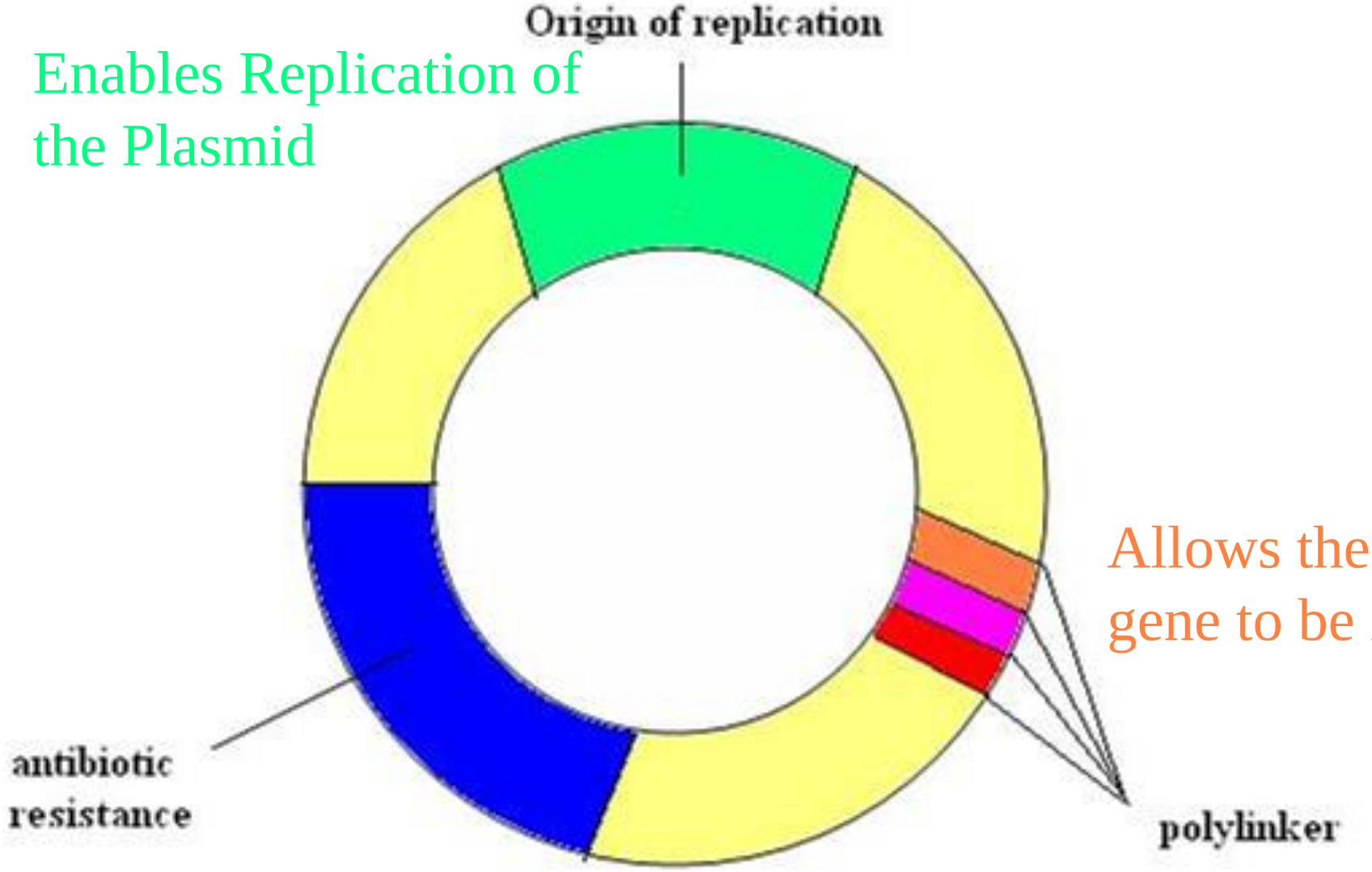






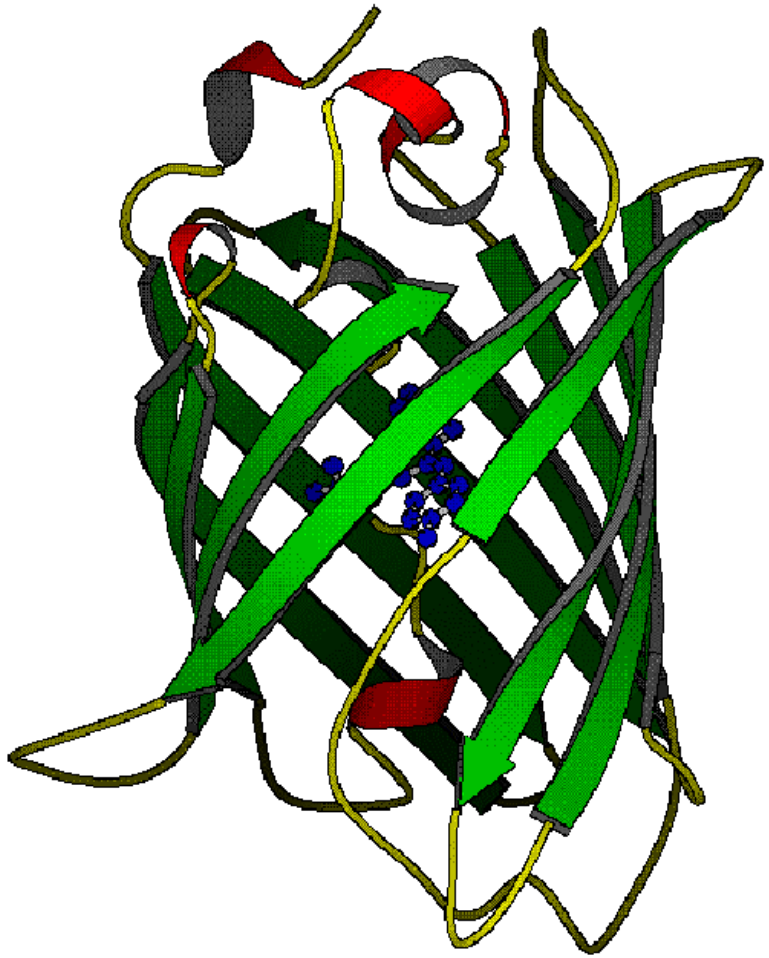
Characteristic of Plasmids

Enables Replication of the Plasmid

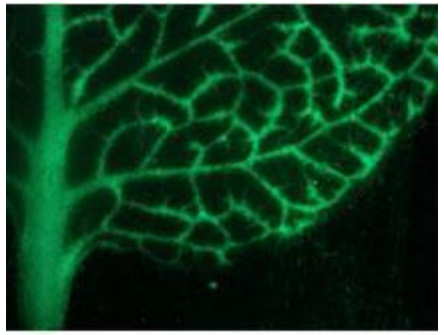


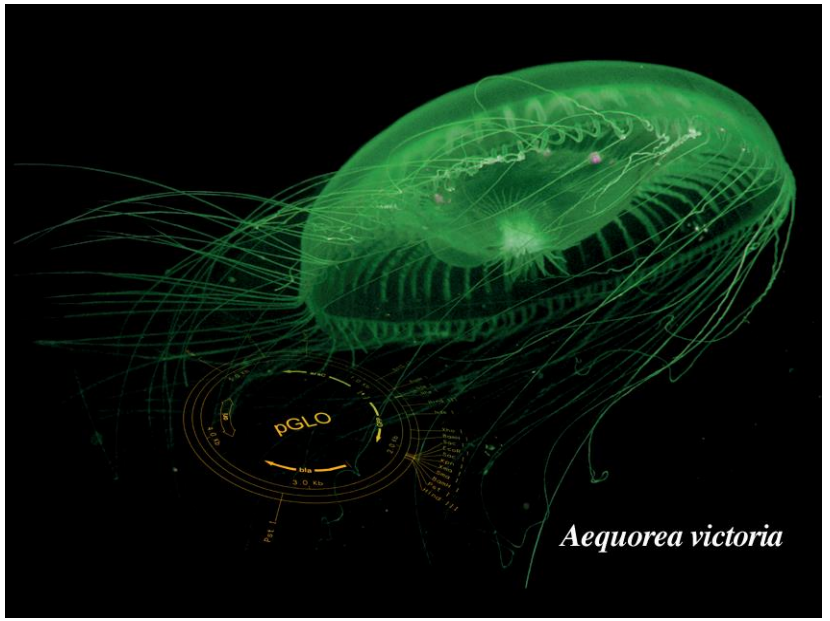
Allows the desired gene to be inserted.

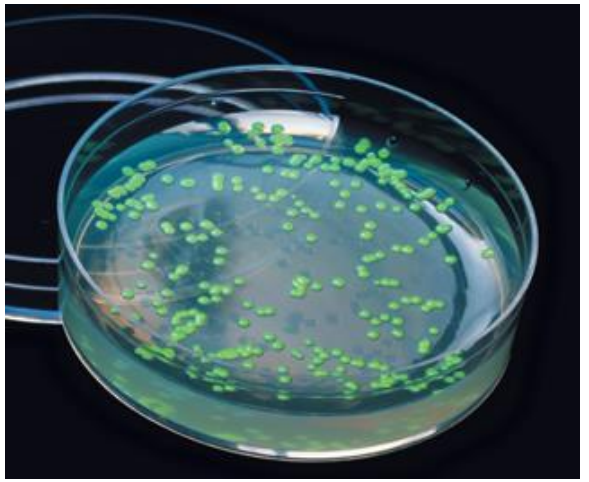
GFP- Green Fluorescent Protein



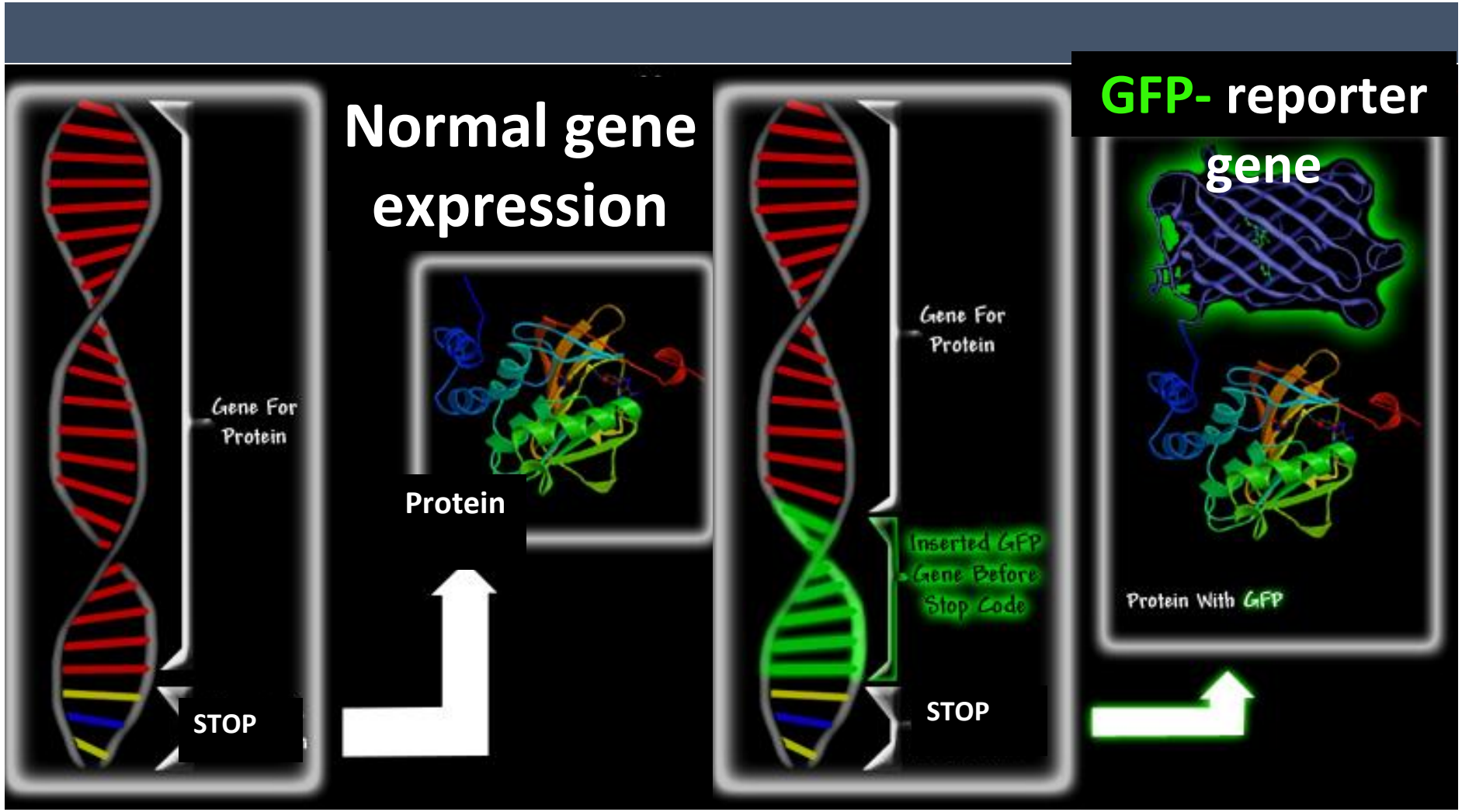
Insertion of Fluorescent Genes







GFP Reporter Gene

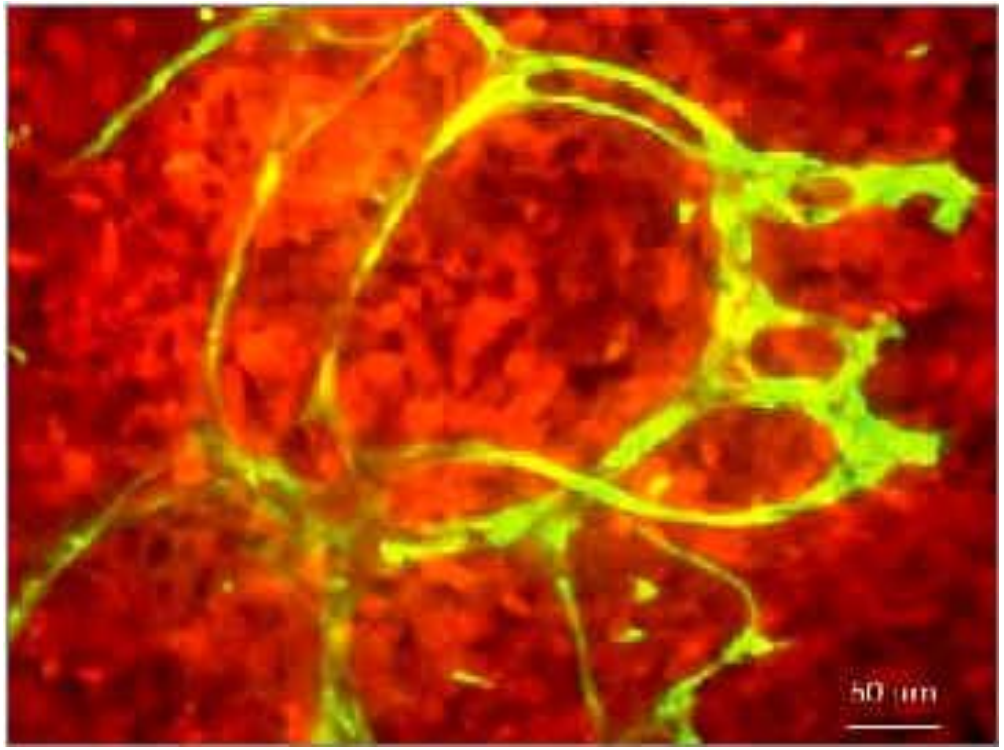


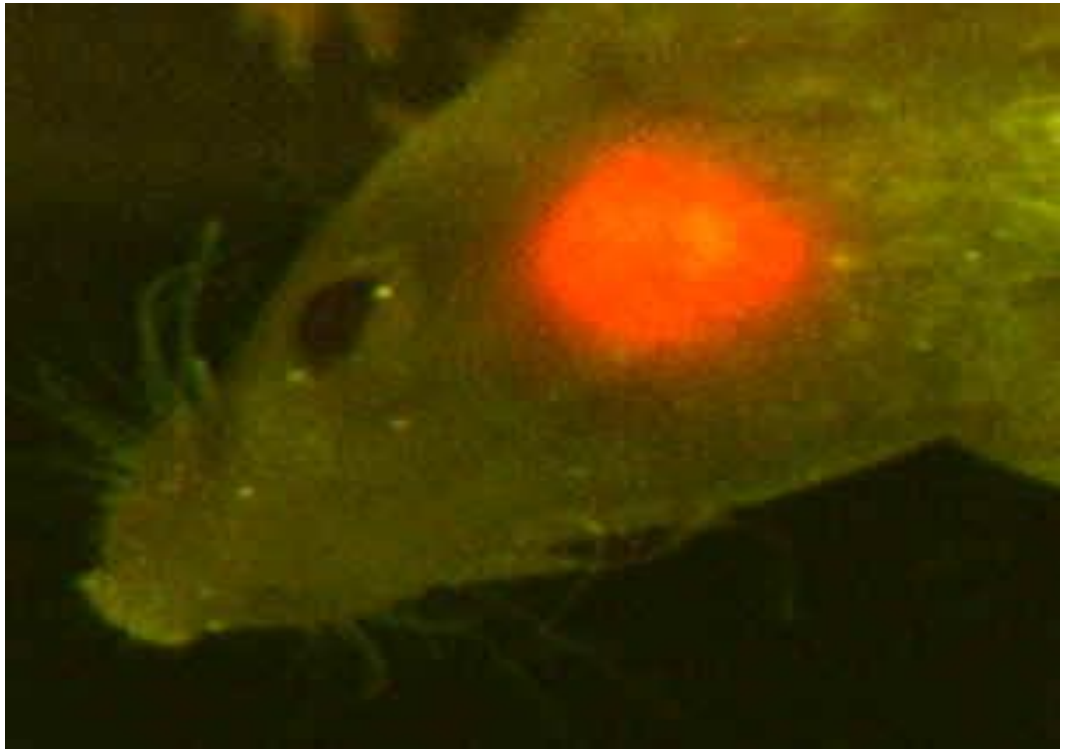
http://www.conncoll.edu/ccacad/zimmer/GFP-ww/prasher.html
With permission from Marc Zimmer

GFP as a Reporter Gene

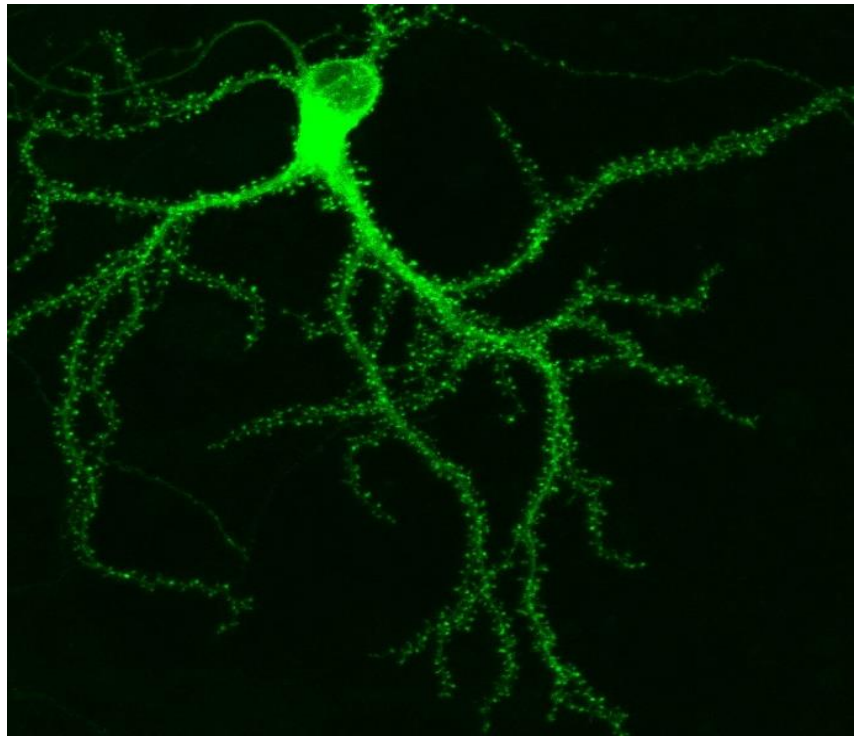
- A visible and defined signal
- Non-toxic protein
- Used for a wide range of research
- Used for research on gene location and expression regulation
- Used for research on protein location within a cell

Cancer and Blood Vessel Research

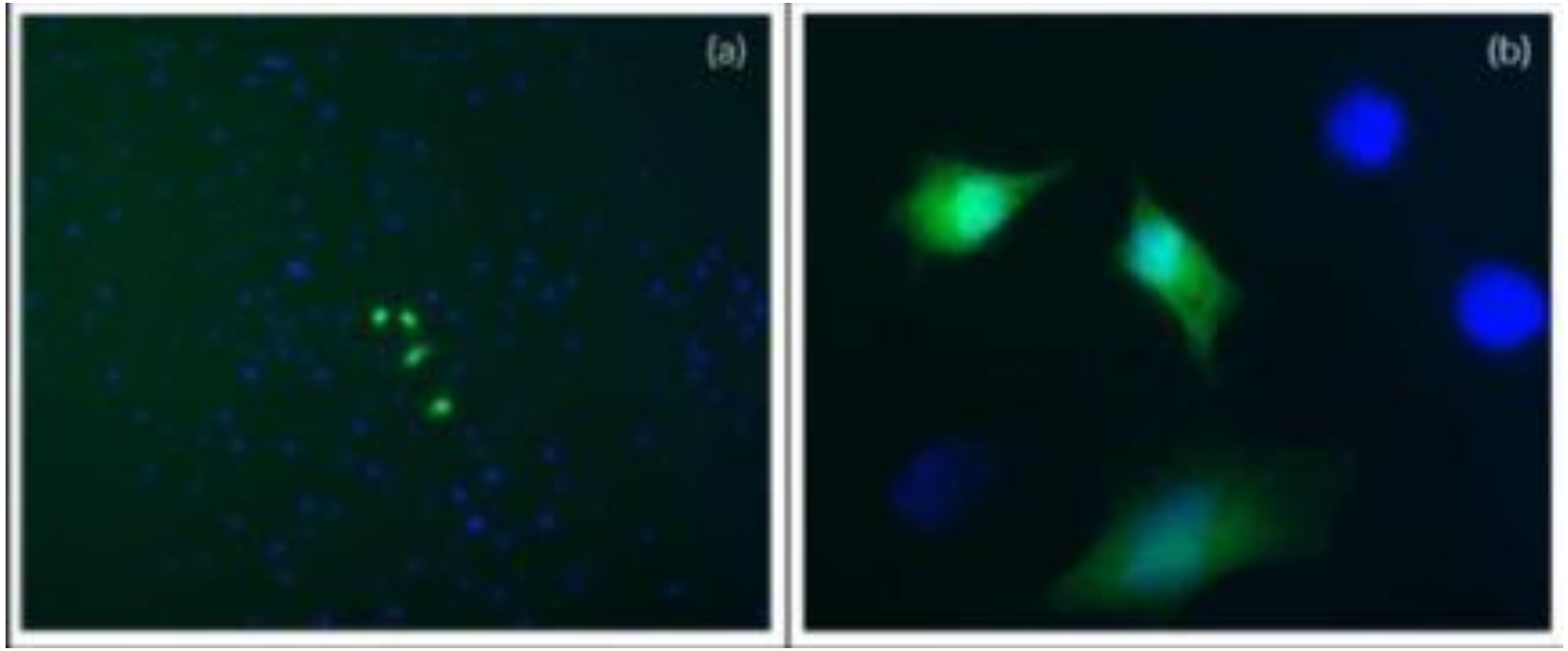




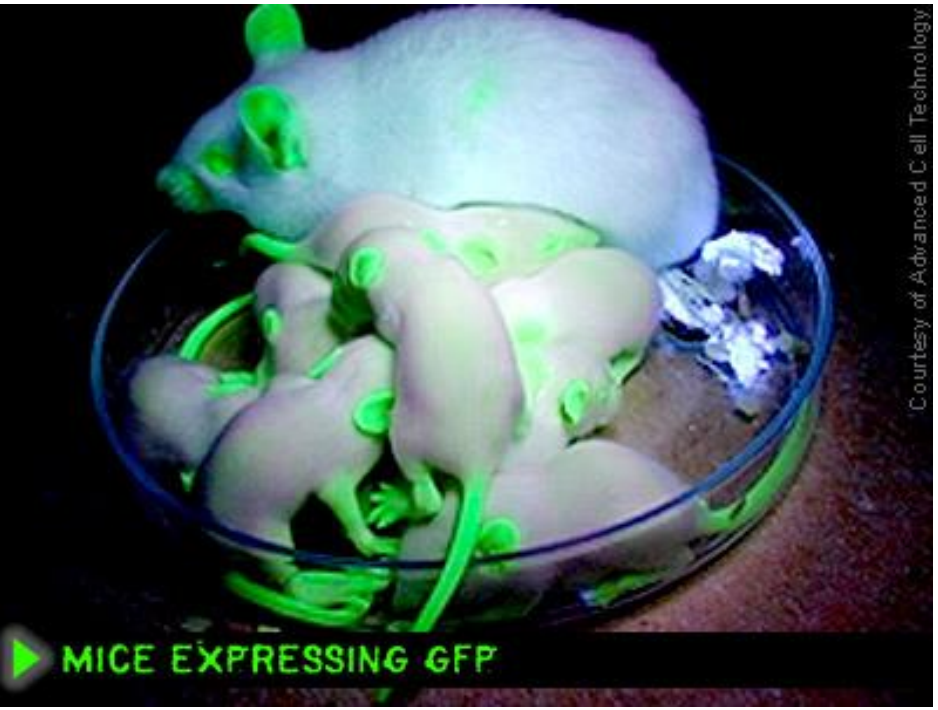
Nerve and Brain Cell Research



Transformation Regulation



Turning Genes on and Off



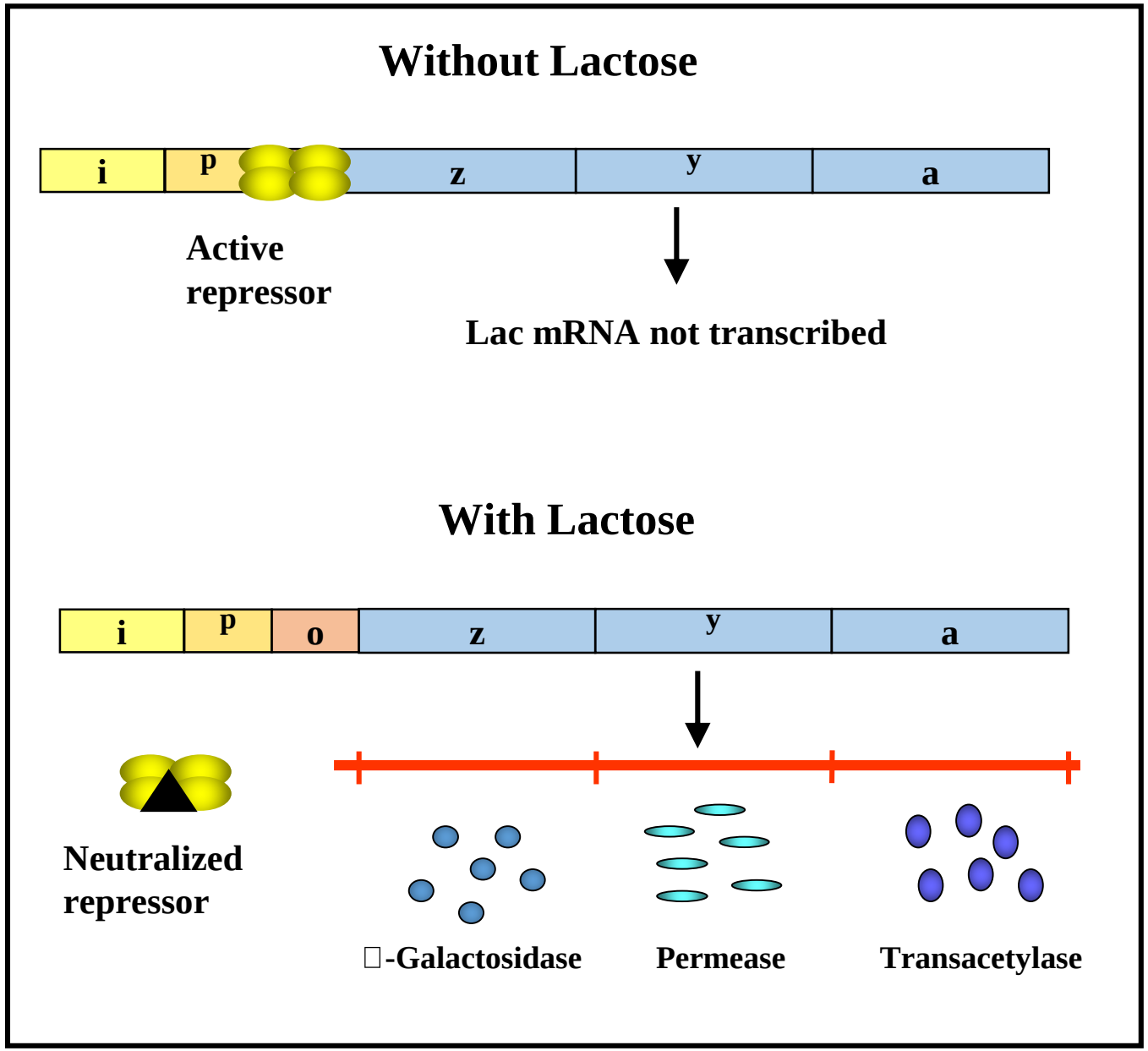
Gene expression types:

Constitutive: The gene is expressed at every stage

Induced: The gene is expressed as a reaction to an environmental signal

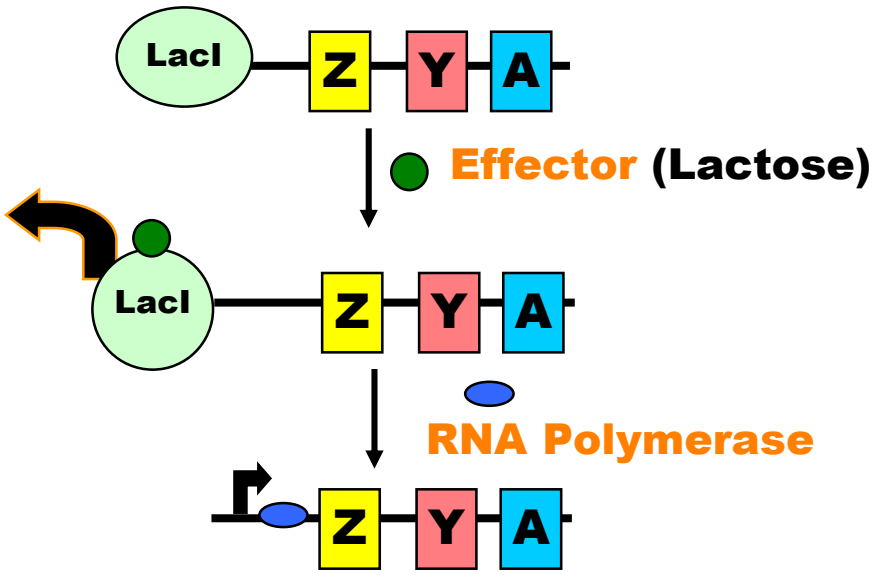
Silenced: No gene expression

The Lac Operon

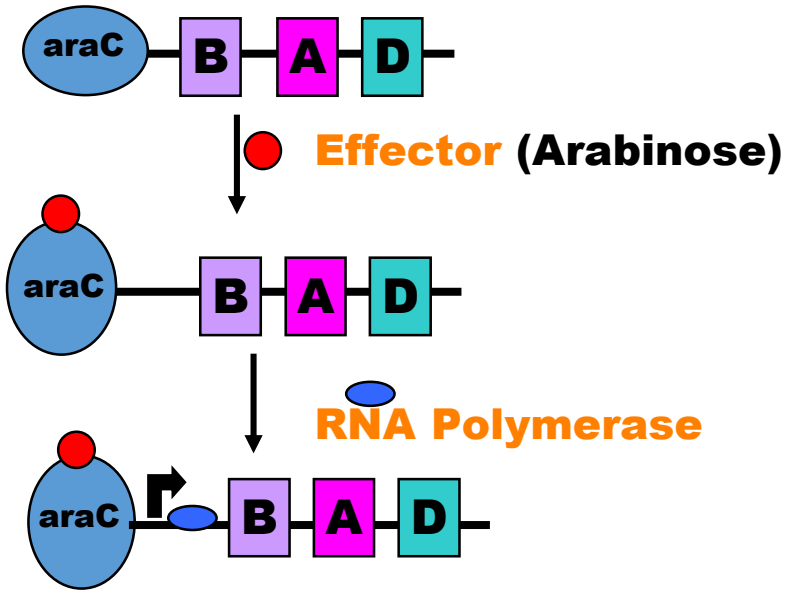


Transcription Regulation

Lactose Operon



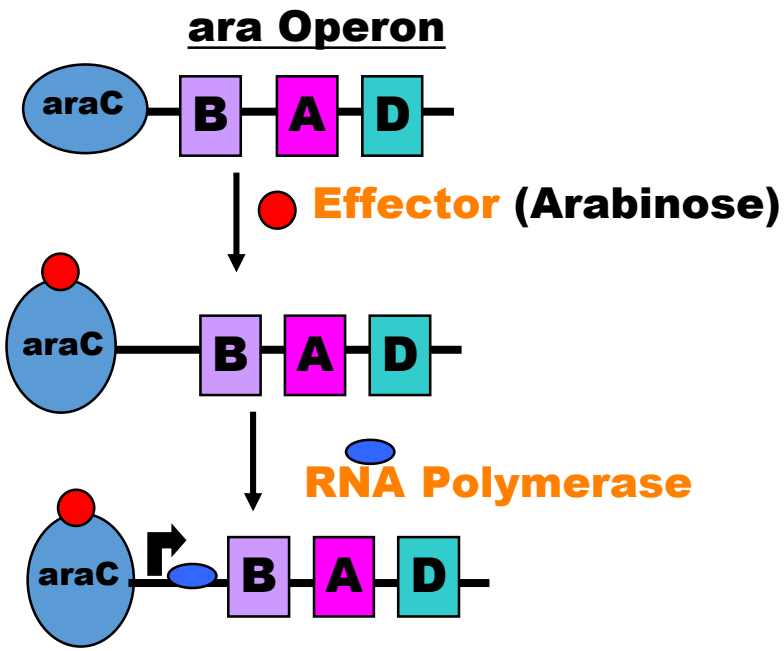
Arabinose Operon

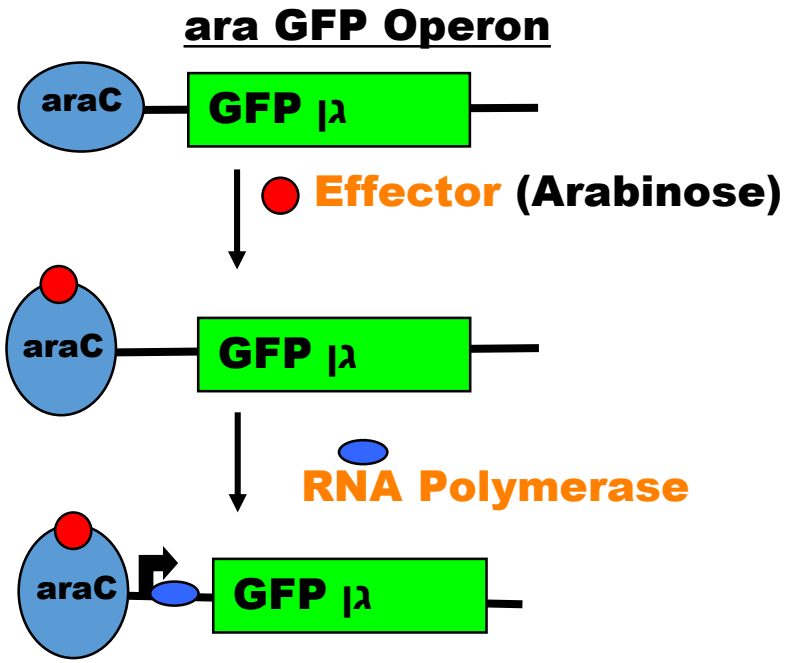


Gene Regulation

Natural Operon

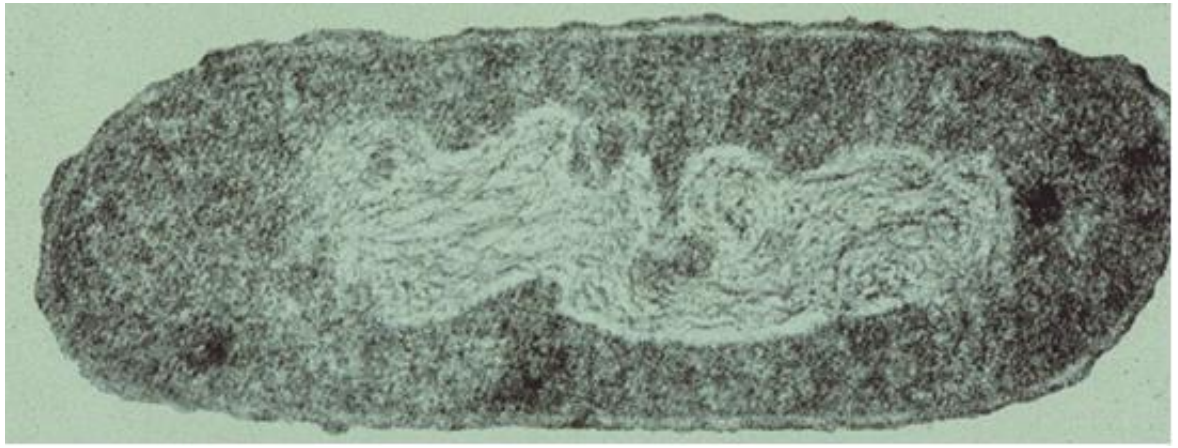
Synthetic Operon



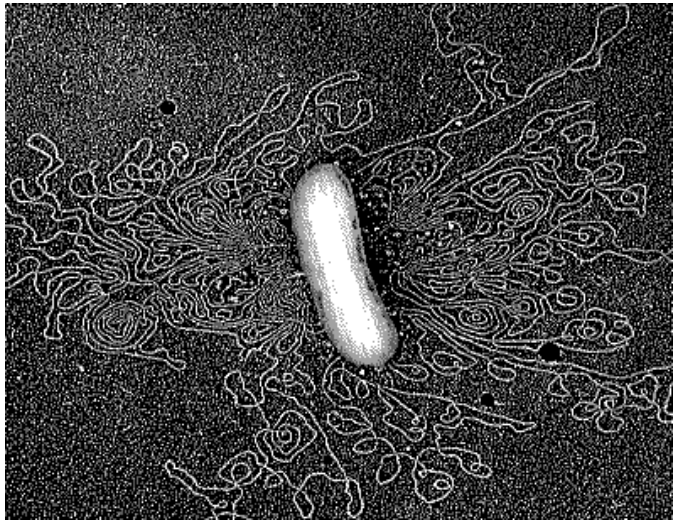


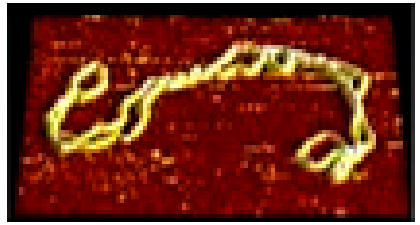
Bacterial DNA

Bacterial Cell



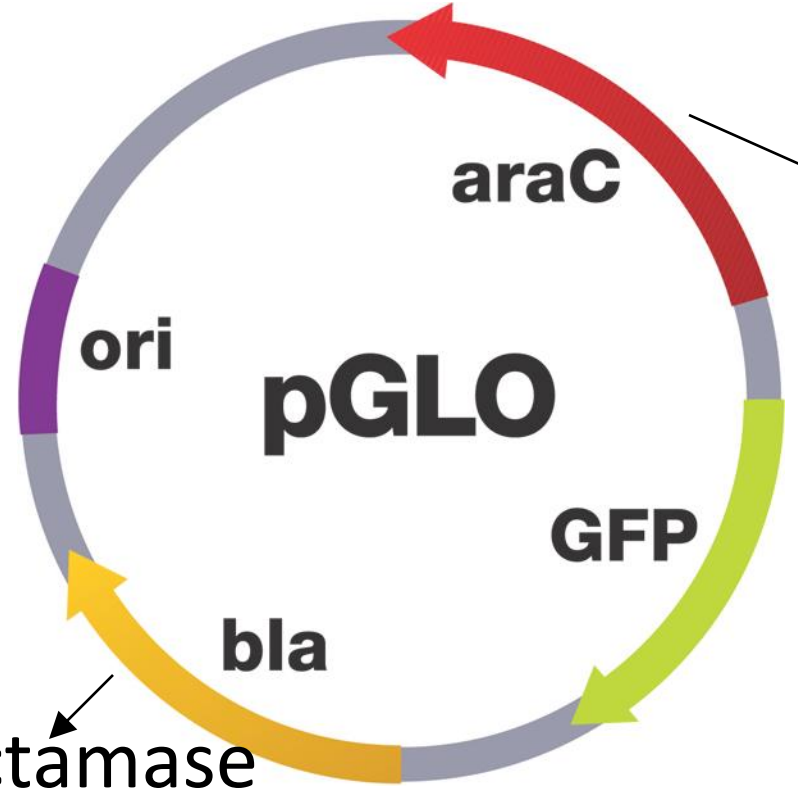
Genomic DNA





Plasmid DNA

Gene Expression in the pGLO plasmid



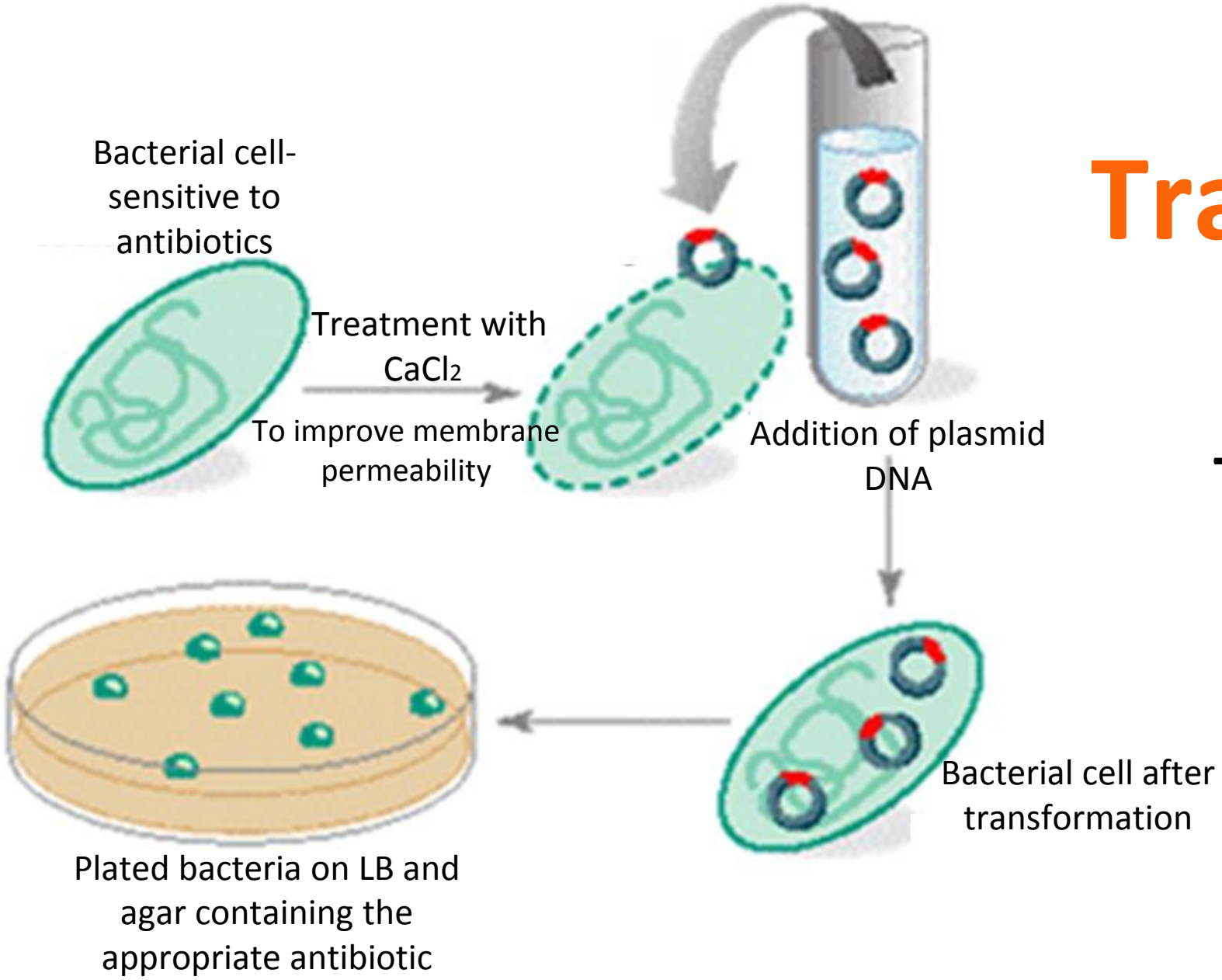
Regulation with Arabinose sugar

GFP

Beta lactamase

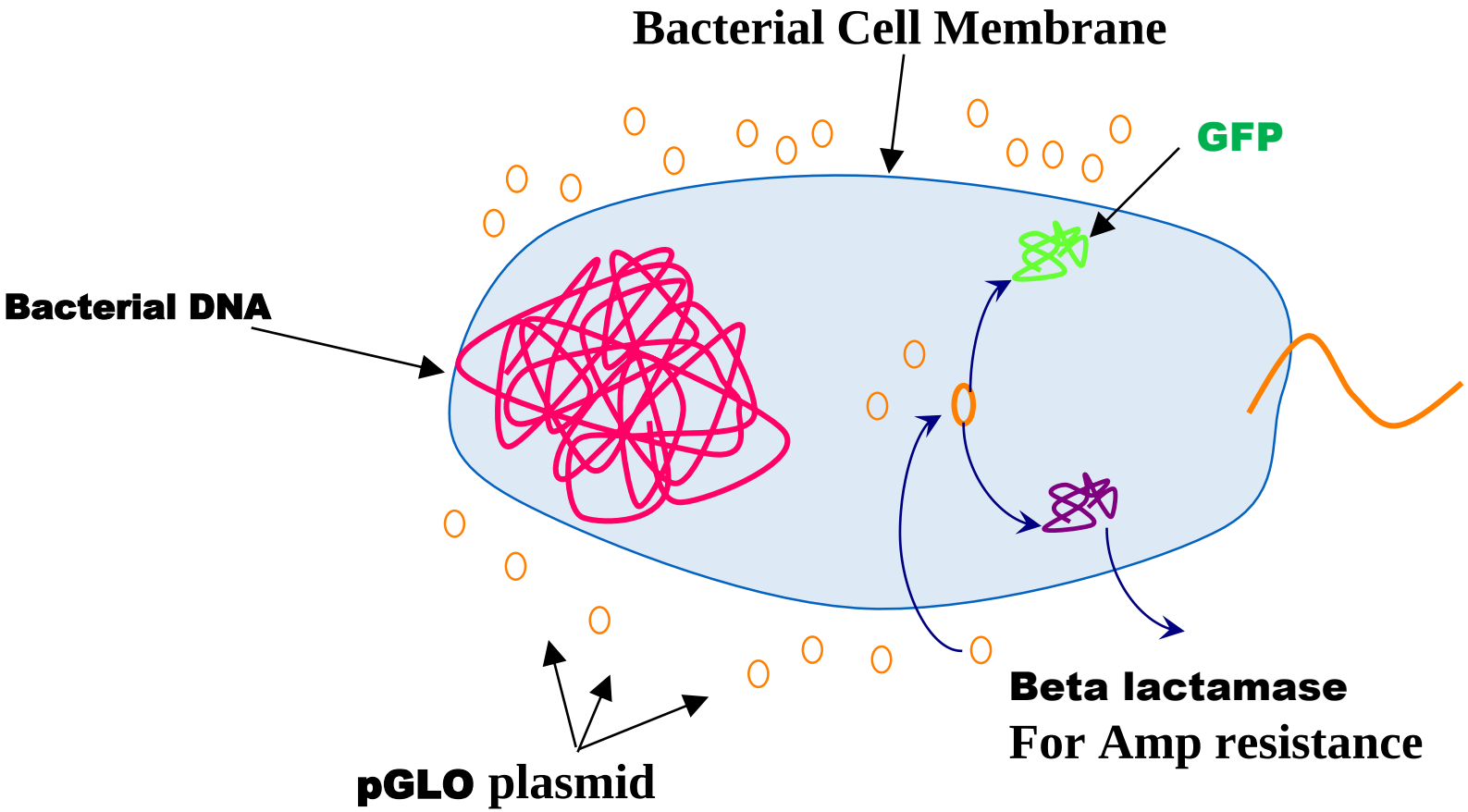
(provides Ampicillin resistance)

What is Transformation?



The insertion of a new gene into an organism

Bacterial Transformation



Transformation Types

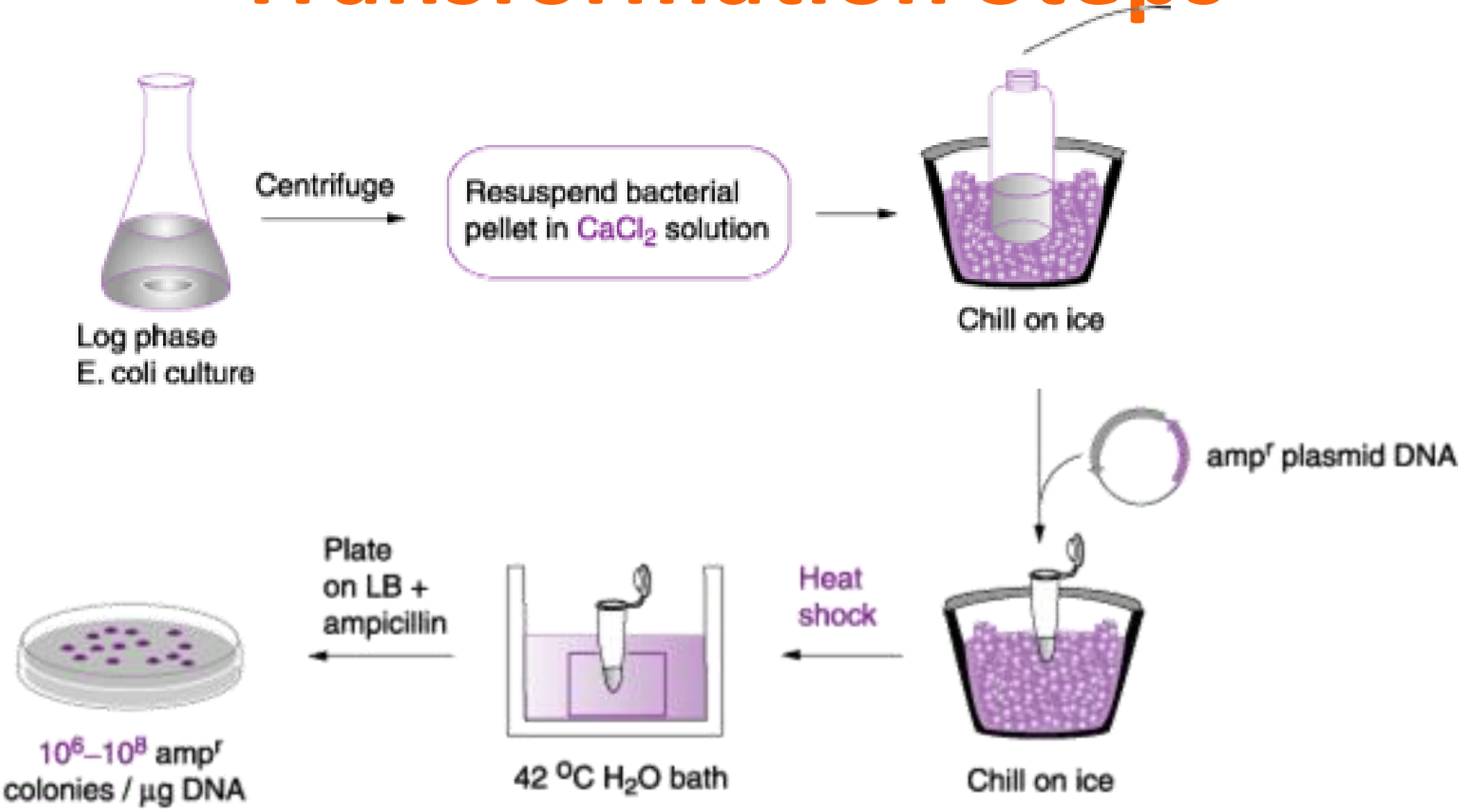
Electroporation

An electric shock causes the membrane to be more permeable to DNA

Heat Shock

Calcium chloride and repeated heat and cold shocks enable DNA to enter the cell.

Transformation Steps



http://www.biochem.arizona.edu/classes/bioc471/pages/Lecture4/Lecture4.html

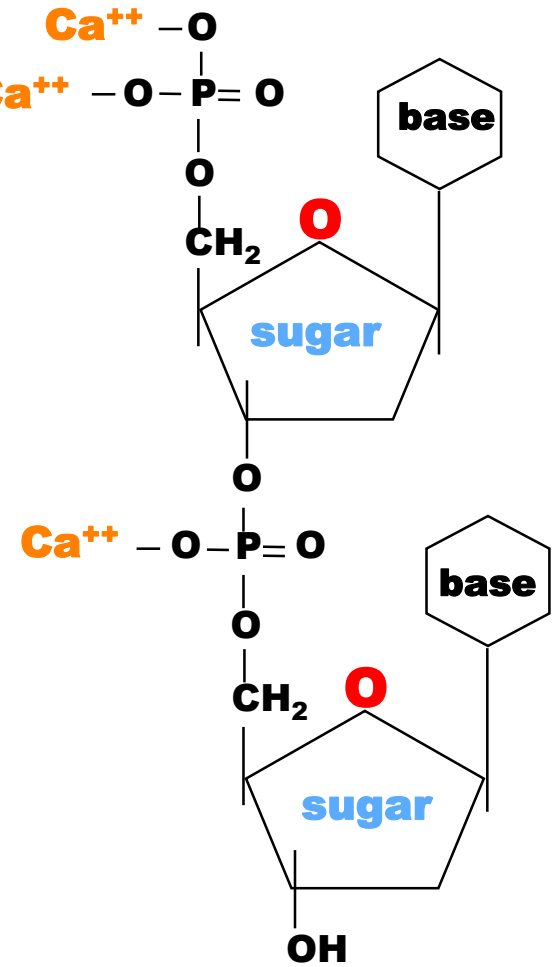
Transformation Steps

- Centrifugation of the cell culture and resuspension in a liquid containing calcium chloride
- Addition of the engineered pGLO plasmid
- Incubate on ice
- Heat shock at 42°C
- Return to ice
- Recovery in liquid LB
- Plate on selective medium

Why do we need these steps?

$CaCl_2$ Solution:

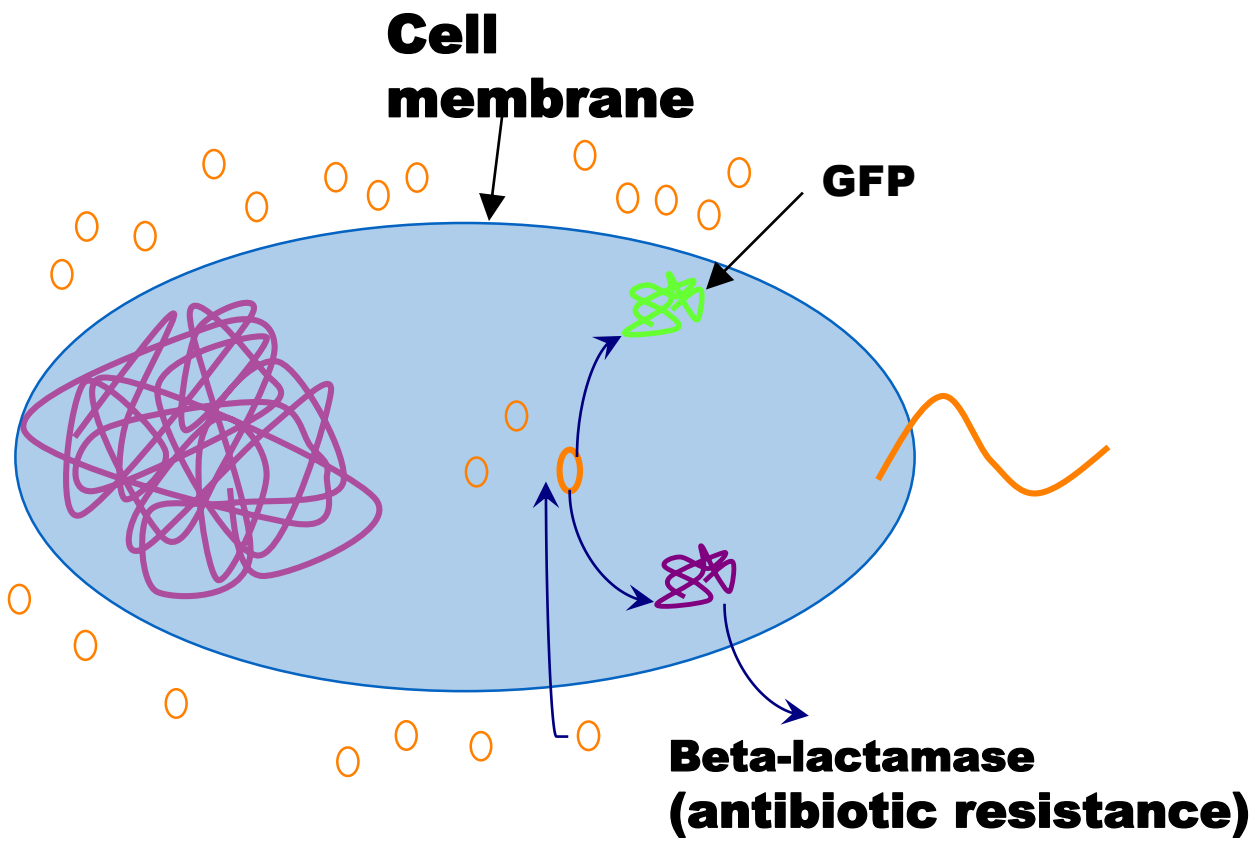
The Ca^{2+} ions bind negatively charged plasmid DNA and make it easier for it to permeate the membrane.



The Transformation Process

Heat:
Increases membrane permeability

Recovery in liquid LB:
Enables expression of the antibiotic resistance gene



LB Medium

Includes all the basic nutrient needed for bacteria:

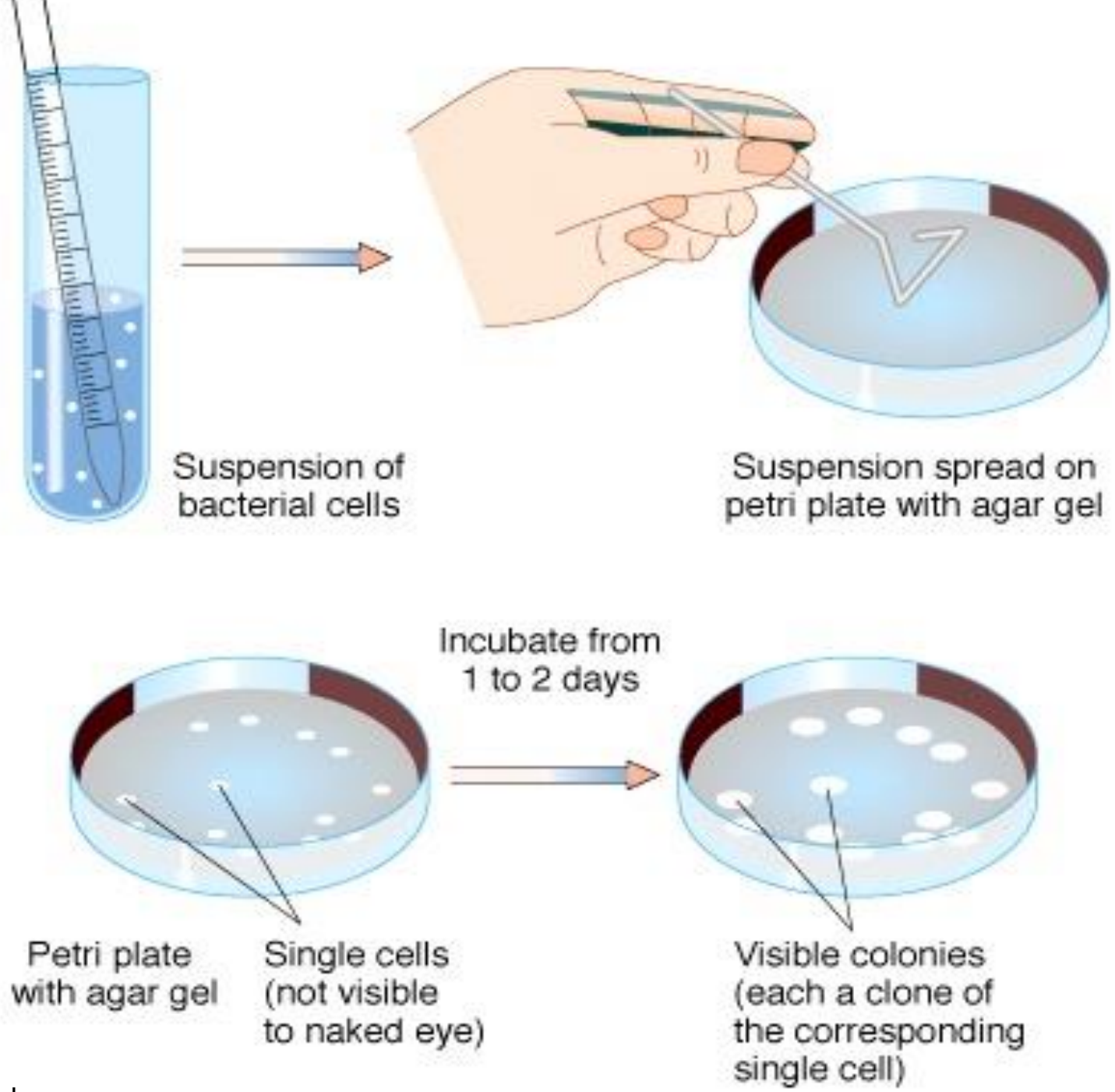
Minerals

Salts

Amino Acids

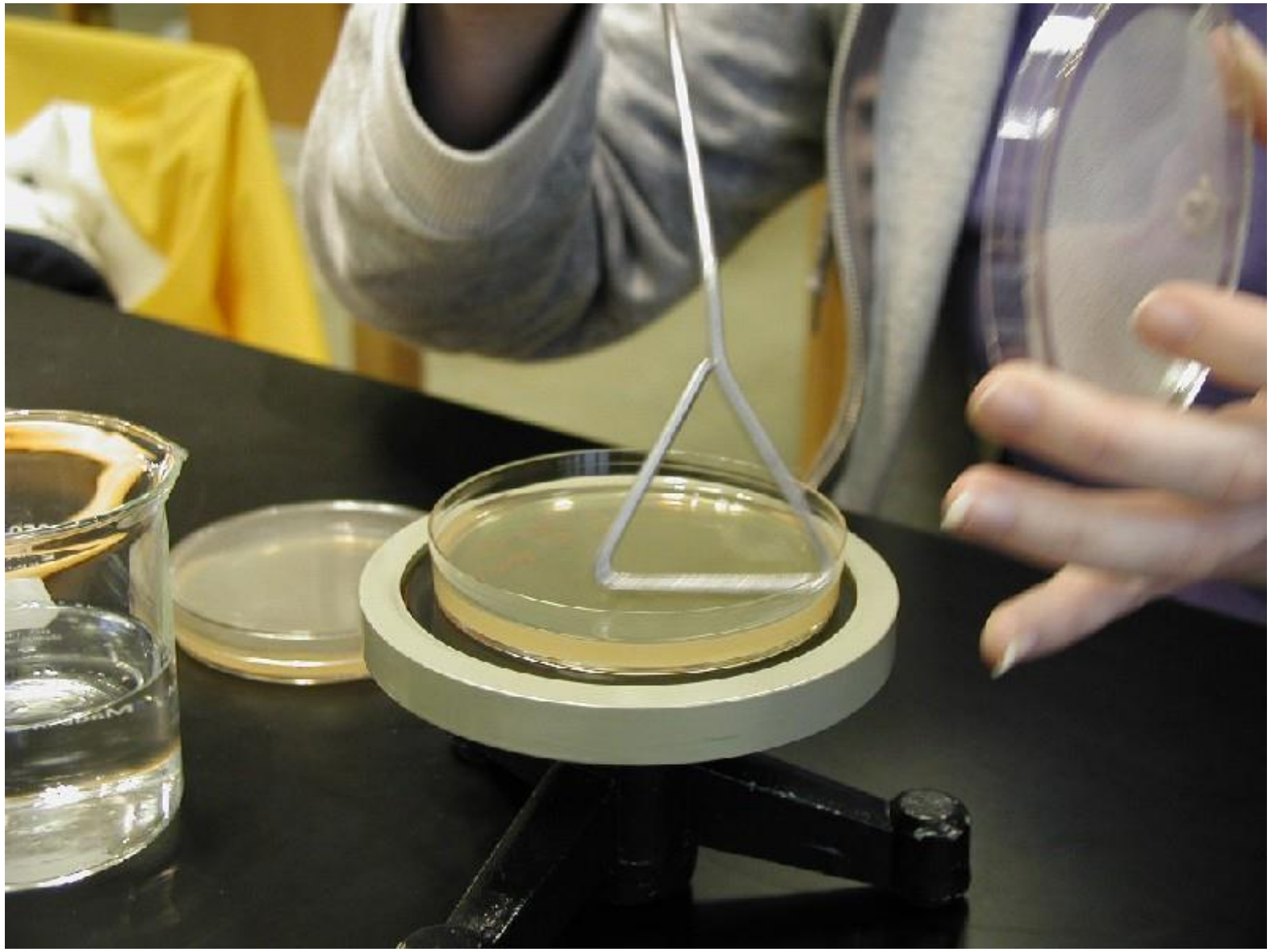
Sugars

Plating Bacteria



http://www.bio.miami.edu/dana/250/250S11_5print.html

Plating Bacteria



Which plate will fluoresce when containing our bacteria?